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Influence of CD4+ Lymphocyte Counts on GB Virus C/Hepatitis G Virus Carriership In HIV-Positive Individuals

Patrick Goubau,* Hsin-Fu Liu, Emmanuelle Goderniaux, and Guy Burtonboy
Virology, Cliniques Universitaires St. Luc, Université Catholique de Louvain, Brussels, Belgium

The duration of the GB virus C or hepatitis G virus (GBV-C/HGV) carriership varies according to the patient group studied. The immune competence of the host may be important. GBV-C/HGV was studied in human immunodeficiency virus (HIV)-infected persons and an attempt was made to correlate the presence of viral RNA or E2 antibodies with CD4+ lymphocyte counts. Of 138 HIV-positive subjects, 30 were GBV-C/HGV RNA-positive and 20 others were E2 antibody-positive, whereas in healthy GBV-C/HGV-infected persons, the proportion of E2 antibody carriers was much higher. On the other hand, a relationship was not found between CD4+ lymphocyte counts and the presence of GBV-C/HGV RNA in the HIV-infected persons. This result does not necessarily imply that the CD4+ lymphocyte count does not affect viral clearance, but the results could be due to the transsectional nature of this study. A longitudinal assessment should clarify this point. ***J. Med. Virol. 57:367–369, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: clearance; viral persistence; homosexual; drug addict; E2 antibody

INTRODUCTION

Recently, two research groups identified a novel flavivirus in humans, which was termed GB virus C [Simon et al., 1995] or hepatitis G virus (GBV-C/HGV) [Linnen et al. 1996]. Despite its name and its distant relationship with the hepatitis C virus (HCV), GBV-C/HGV is unlikely to be a frequent cause of hepatitis [Alter, 1996]. In contrast to HCV, this virus is very stable with a low rate of mutations [Nakao et al., 1997]. It has been shown that a number of persons have antibodies to the E2 envelope protein of the virus [Tacke et al., 1997]. Most of these individuals lack GBV-C/HGV RNA, suggesting that the presence of antibodies is a sign of virus clearance. In persons with hemophilia, the prevalence of GBV-C/HGV is much lower than that of HCV, whereas in blood donors GBV-C/HGV RNA is more often present [Jarvis et al., 1996]. This has been seen as an indication that most patients clear the virus after infection. However, in other patient groups, for example, hemodialysis patients, persistence of the infection over many years seems the rule [Cornu et al., 1997]. Therefore viral persistence may be related to the immune status of the host.

We investigated human immunodeficiency virus (HIV)-positive persons for signs of GBV-C/HGV infection and whether viral RNA carriership was related to their CD4+ lymphocyte level, as a measure of their immune competence.

MATERIAL AND METHODS

Serum samples of 138 HIV-positive individuals were collected retrospectively. The samples had been stored for a variable time at –80°C. For 110 of the 138 cases, the CD4+ lymphocyte count was known at the time of sampling. The patients were divided into several categories according to their possible mode of acquisition of HIV (see Table I).

Viral nucleic acids were extracted from the samples with the High Pure Viral Nucleic Acid kit (Boehringer Mannheim) following the manufacturer's instructions, and cDNA synthesis was carried out by the MMLV reverse transcriptase (Gibco BRL, Bethesda, MD) using random hexamer primers.

GBV-C/HGV RNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) in two different genomic regions (NS5 and 5′ noncoding region) as described previously [Cornu et al., 1997; Liu et al., 1998]. The amplification in the 5′ noncoding region was by nested PCR.

All samples were tested serologically for E2 antibodies using the μ Plate Anti-HGenv kit (Boehringer Mann-

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*Correspondence to: Prof. P. Goubau, Virology, Cliniques Universitaires St. Luc, Ecole de Santé Publique 3055, 30 Clos Chapelle-aux-Champs, B-1200 Brussels, Belgium. E-mail: goubau@mblg.ucl.ac.be

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TABLE I. Prevalence of GBV-C/HGV Infection in Different HIV-Infected Groups

Group	*n*	GBV-C/HGV RNA+	E2 antibody +	Total GBV-C/HGV	%	95% CI
Heterosexual Caucasian	15	2	2	4	26.7	7.79–55.10
Heterosexual African	59	11	9	20	33.9	22.08–47.39
Intravenous drug user	22	8	4	12	54.5	32.21–76.61
Sexual partner of IV drug user	5	1	0	1	20.0	0.51–71.64
Transfusion recipient	18	2	1	3	16.7	3.58–41.42
Homosexual man	19	6	4	10	52.6	28.86–75.55
Total	138	30	20	50	36.2	28.40–44.50

GBV-C, GB virus C; HGV, hepatitis G virus; HIV, human immunodeficiency virus; CI, confidence interval; IV, intravenous.

heim, Mannheim, Germany) following the manufacturer's instructions. All positive results were confirmed as prescribed by differential testing with and without the E2 antigen.

Confidence intervals on proportions were calculated and the chi-square test used for comparison of proportions. Means of $CD4^+$ lymphocyte counts were compared with the unpaired *t*-test and two-tailed *p* values (GraphPad Prism 2.0, San Diego, CA).

RESULTS

Overall 20 (14.5%) individuals were anti-E2 positive. Five were positive for GBV-C/HGV RNA by nested PCR only and 25 were positive with both PCRs, giving a total of 30 (21.7%) RNA positives. No one individual was positive for both RNA and antibodies. Overall, the prevalence of past and present GBV-C/HGV infection was 50/138 (36.2%; 95% CI: 28.4–44.5). The proportions of antibody positives or RNA positives were not significantly different from a 50/50 proportion. The prevalences in the different groups are given in Table I. Among Caucasians, 42.5% (95% CI: 30.7–55.2) had been infected by GBV-C/HGV, versus 28.6% (95% CI: 17.3–42.2) of Africans. The higher percentage among Caucasians, although not significant on the numbers studied, can be explained by the high proportion of intravenous drug abusers and homosexual males in this population group. The difference in prevalence in the different groups did not reach the level of significance.

In 110 HIV-infected patients, the $CD4^+$ lymphocyte count at the time of sampling was known. Sixteen had E2 antibodies and 23 GBV-C/HGV RNA. The mean count of $CD4^+$ lymphocytes/μl in the 16 antibody positives was 360.5 (SEM = 93.5) and 301.2 (SEM = 33.4) in the 23 RNA positives (difference not significant), whereas the mean count was 277.1 in the 71 GBV-C/HGV uninfected persons. When grouping the GBV-C/HGV patients according to the $CD4^+$ lymphocyte status, we found 7 RNA positives and 5 antibody positives in those with more than 400 cells/μl. The respective figures for those with less than 400 cells/μl were 16 and 11. No differences were thus obvious between these two groups. With a cut-off level of 100 $CD4^+$ lymphocytes/μl, 20 patients above the level were GBV-C/HGV RNA positive and 13 were E2 antibody positive, while under this level the respective figures were 3 and 3.

DISCUSSION

After infection with GBV-C/HGV, a number of individuals clear the virus when anti-envelope antibodies (directed against an E2 epitope) appear, whereas other individuals will remain carriers for a variable period [Dawson et al., 1996; Jarvis et al., 1996; Nakao et al., 1997]. In the clearance of GBV-C/HGV, the immune status of the host may be important and could explain the differences in apparent persistence of the viral RNA in different patient groups. HIV-infected individuals will go through different degrees of immune depression during the course of their infection. A measure of their immune status is the level of $CD4^+$ lymphocytes. For this reason, an attempt was made to correlate their $CD4^+$ lymphocyte level with the presence of GBV-C/HGV RNA or E2 antibodies. Overall, 50/138 (36%) had been infected with GBV-C/HGV. Among these, the $CD4^+$ lymphocyte status at the time of sampling was known in 39. The mean $CD4^+$ lymphocyte counts were not significantly different between the 16 antibody positives (360 $CD4^+$/μl) and the 23 RNA positives (301 $CD4^+$/μl). This finding contrasts with the study of HIV carriers by Heringlake et al. [1998], who found higher mean $CD4^+$ lymphocyte counts in GBV-C/HGV RNA-positive patients than in E2 antibody carriers or uninfected patients. Their study suggested that GBV-C/HGV might be a favorable prognostic factor in HIV infection. Looking differently at our data, differences were not observed in the proportion of RNA positives among infected subjects according to their $CD4^+$ lymphocyte level. Thus, GBV-C/HGV clearance did not correlate with present $CD4^+$ lymphocyte concentrations. This finding does not mean that immune status is not important at the moment of clearance. This study was cross-sectional and therefore cumulated the effects of past and present situations. A longitudinal follow-up of HIV and GBV-C/HGV co-infected patients would be necessary to solve this question.

In a study by Nübling et al. [1997], subjects who were positive for both E2 antibodies and GBV-C/HGV RNA, were also HIV positive. In our study group of HIV -infected individuals, we did not see any person who was positive for both. This was also the case in the study of Heringlake et al. [1998]. Among the HIV-infected subjects in our study, 20 were positive for E2 antibodies and 30 for GBV-C/HGV RNA. This finding is not statistically different from a 50/50 proportion. In general

population studies, approximately five times more individuals are positive for E2 antibodies than for GBV-C/HGV RNA [Tanaka et al., 1998], whereas in renal failure patients, this proportion tends to be equal [Szabo et al., 1997], as in our study. This finding might indicate that a certain degree of immunosuppression is indeed responsible for the lack of clearance of the virus.

A high prevalence of infection was found among HIV-infected individuals, suggestive of common transmission routes or risk factors. High and similar percentages of infection were found in male homosexuals (53%) and in intravenous drug abusers (55%). The prevalence of the virus in these two groups explains the higher rates among Caucasians (42.5%), who include all individuals from these two groups, when compared with Africans (28.6%). In other studies, the infection rate in male homosexuals was lower than in intravenous drug abusers, indicating that sexual transmission was less efficient than parenteral transmission [Stark et al., 1996; Nübling et al., 1997]. The fact that we selected only those individuals who were HIV positive biased the subject population toward high-risk activities, which might also have led to a higher rate of GBV-C/HGV infection.

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